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**RAPID TOXICITY DETECTION IN WATER QUALITY CONTROL UTILIZING AUTOMATED
MULTISPECIES BIOMONITORING FOR PERMANENT SPACE STATIONS**

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ABSTRACT

Automated biomonitoring may provide real-time physiological response information as a result of cause/effect relationships between developing toxicity and representative aquatic animals. However, since the reliability of using information from a single-species or from selected water quality measures may be subject to question when viewed in light of developing toxicity, we developed a computer-assisted multiple species biosensing system for water quality monitoring. Because fish have typically been used in earth-based applications of automated biomonitoring and are assumed to be difficult to maintain in near zero gravity, emphasis was placed on developing methods for detecting species-specific bioelectric potentials induced by unrestrained mussels and other sedentary invertebrates. A specially designed differential amplifier was constructed for measuring signals induced by various activities from not only fish but invertebrate subjects. Specific responses were detected as discrete analog signals, each sign wave converted to a digital voltage, and filed in computer storage. A management program provided various means for data gathering, filing, and retrieval.

The objective of this study was to evaluate proposed design characteristics and applications of automated biomonitoring devices for real-time toxicity detection in water quality control on-board permanent space stations. Simulated tests in downlinking transmissions of automated biomonitoring data to earth-receiving stations were simulated using satellite data transmissions from remote earth-based stations.

INTRODUCTION

Computer-assisted automated biomonitoring systems used to detect developing toxicity in water quality surveillance programs have typically relied upon a single species, the fish, as the test subject. In a review by Cairns and

van der Schalie (ref. 1), representative early warning biological monitoring systems employing fish as biosensors and several innovative systems designed to measure invertebrate responses to water quality change were evaluated. From those described for detecting aquatic invertebrate movement activities, several devices were identified which included remote probe configurations for receiving action potentials generated when the animal's body integument changed positions (ref. 2). Applying similar techniques for water pollution monitoring, Idonlboye-obu (ref. 3) established a bioassay procedure where marine decapod action potentials could be detected under increasing exposure to toxic hydrocarbons. Incorporating a computer for data management, Macioroaski, et al. (ref. 4) evaluated crayfish abdominal movements as a measure of increasing water toxicity to aquatic animals.

Although automated biomonitoring devices may prove effective in detecting developing toxicity in certain specific applications, it seems unreasonable to expect a single-species or animal group to be universally responsive to the wide variety of pollutants which might contaminate space station water supplies. Recognizing the complex matrix of abiotic and biotic interrelationships and the episodic character of many closed water supply systems, the relative toxicity of certain substances may be modulated either through attenuating or amplifying processes. Therefore, to account for possible toxic modulatory effects and the bias of depending on a single-species for judging toxicity, the development of an automated multiple-species biological monitoring system employing groups of different types of aquatic animals representing various trophic levels could prove helpful to space station water management and quality control programs. The initial research and design for an automated multispecies biosensing device coupled with physical sensors capable of simultaneously deriving biotic response data and physical parameters has been discussed by Morgan and Young (ref. 5) and Morgan, Young, and Crane (ref. 6).

In meeting the objective of developing a computer-assisted multispecies biological monitoring system capable of giving an early warning of developing toxicity in water supplies, emphasis was placed on the following: (1) extending system versatility by developing techniques for detecting responses from a variety of aquatic invertebrates occupying different trophic levels, (2) developing a universal differential amplifier capable of detecting bioelectric responses from an array of different aquatic animals, (3) expanding amplifier and register interface units from groups of discrete in-put channels and coupling these with IBM-PC based systems, and (4) integrating these components and in-putting biological response information into a data collection platform for simulating downlinking to earth-based receiving stations.

SYSTEM DESIGN

BIOLOGICAL CONSIDERATION

Crucial to this effort was the observation that various unrestrained aquatic animals induce bioelectric signals into the surrounding water which can be recorded as rhythmic analogue signals representative of specific movement activities, i.e. gill beats, heart rates, etc. Utilizing appropriate techniques

and accompanying electronics, changes in bioelectric responses may be detected and processed with respect to possible water quality induced stress to the animal.

HOLDING CHAMBERS FOR ANIMALS

Design characteristics and specific configurations used for fish, mussel, and burrowing mayfly nymph maintenance while monitoring have been discussed previously (ref. 4, 5). In summary, housing individual free-swimming fish chambers consisted of PVC plastic tubes equipped with a pair of probe-antennae. Probes were designed to receive bioelectric potentials induced by each gill ventilatory response and were connected to appropriate electronics for detection. (Figure 1). Burrowing mayfly nymph chambers consisted of selected lengths of tygon or plastic tubing of various diameters, each configured with a set of micro-antennae. Once the nymph was established within the artificial tube chamber, bioelectric signals generated by the rhythmic peristaltic oscillation of its paired abdominal gills were easily detected by accompanying electronics.

Mussel myoelectric events were readily monitored by micro-probe antennae which were located between the mantle tissue and the inner valve of the animal. Caution was taken not to insert these into the tissue. Combined with gill action, foot muscle, and abductor muscle events, bioelectric responses generated by peristaltic contractions of the heart could be selected for and recorded.

A multiple species complement was realized by simultaneously monitoring groups of bluegill sunfish, heelsplitter mussel, and burrowing mayfly nymph artificial tube chambers. Artificial tube chambers containing individual nymphs were positioned in a subcontainer which was floated at the surface of the tank holding the mussels and fish chambers. Groups of four free-swimming individuals from each species were isolated from each other and electrodes connected to individual channels for data entry to the minicomputer.

COMPUTER-ASSISTED AUTOMATED MONITORING

Differential d. c. amplifiers were constructed that meet requirements for a wide range of water quality conditions and were flexible enough to detect weak bioelectric events from various aquatic animals (ref. 5). One amplifier was used for each animal and the gain and filter set to read the specific analog frequencies. Undesirable high frequencies were filtered at the initial stages of amplification. Amplifiers were interfaced to an IBM-PC compatible having various peripherals, including tape backup and modem. Analog response signals were digitized and stored in registers until inputted to the computer for filing on disk. The complete automated computer-assisted biomonitoring system (ACABS) was an updated version of the automated fish respiration monitoring system (AFIRMS), which was fabricated by the Data Services Branch, TVA (ref. 6).

SYSTEM TESTING

PREPARING ANIMALS FOR MONITORING

Using a continuous monitoring configuration, fish were individually isolated in plastic tube chambers which were placed in glass aquaria equipped with flow-through water exchange. Mussels were positioned on gravel substrates within the aquaria. Because mayfly nymphs displayed a tendency to abort artificial burrows made of tygon tubes, they were further isolated by placing an individual tube chamber housing a single nymph in a screen-bottomed plastic cylinder. This isolation cylinder was then submerged in the aquaria. Test subjects were allowed to acclimate for a week or more in isolation while receiving once-through lake water and prevailing photoperiods. Animals received food while acclimating to ambient conditions but not during test treatments.

BENCH TESTING MONITOR

Preliminary testing was done by selecting groups of acclimated individuals from each of three groups, i.e., bluegill, burrowing mayfly nymph, and heel-splitter mussel and continuously monitoring their bioelectric frequencies for a one- to three-day reference period. Then, two individuals from each group were selected as controls for a one-day treatment interval which followed. A one-day treatment was designed to simulate an acidic water supply where a pH depression between 5.5 and 4.5 was gradually achieved over several hours period. The initial ambient pH value ranged from 7 to 0. Treatment solutions were batch mixed lake water and were administered by interrupting reference lake water flows and allowing treatments to gradually replace ambient reference flows. Exposure to maximum pH depress persisted for 6 to 12 hours when reference lakewater was again delivered without altering flow rates. Following the acid exposure treatment, reference ambient conditions were reached within several hours while test subjects were continually monitored for the remaining few days of the recovery period.

Prior to acid treatments, a time-rated data base of bioelectric responses was developed from test fish maintained under ambient once-through lake water flows. This data base was then used as a reference for comparing changing stressful responses induced by acid water exposures (Figure 2).

DATA CAPTURE VIA SATELLITE

Referred to as a remote data collection platform (CDP), this self-contained computer which functions as a d. c. powered central controller and processor in conjunction with various communication modeules was used to simulate data capture via satellite downlinking. Features include expandable PROM/RAM memory, a programmable calendar/alarm clock, an S-34 controller port, RS-232 operator port, and being supported by the S-FORTH operating system.

Breathing events generated by individually monitored fish, for example, were received by the DCP in digital form. The data were held for a pretest monitoring interval by interface registers prior to submission to the DCP. Data presented to the DCP were transmitted to the NOAA-Geostationary Operational Environmental Satellite (GOES) six times daily. Broadcast data received by GOES were then retransmitted to a receiving station for processing (Figure 3). Depending on monitoring needs, simultaneous transmission of water quality parameters included: temperature, dissolved oxygen (DO), hydrogen ion concentration (pH), specific conductance, and oxidation-reduction (redox) potential. Physical sensors were positioned in-stream alongside fish-holding chambers.

PROPOSED WATER SOURCE TOXICITY TESTING

Coupled with existing methods for biological and chemical water quality testing of space station water supplies, automated biosensing may have merit as an early warning to developing problems. Such a protocol would eliminate the inherent weaknesses of relying on chemical/physical measures alone. Unlike most other biological test systems, the automated biomonitoring approach provides real-time detection of developing toxicity, continuously (ref. 1, 6).

CONCLUSION

Through previous studies using remote biosensing devices for water quality monitoring, a series of technical questions have surfaced which will require attention should automated biomonitoring be considered as part of an early detection to developing toxicity in water sources on permanent space stations. Specifically, even though fish have been used for more than a decade as test subjects in earth-based automated biomonitoring systems, little information is available on the effect of near zero gravity on fish breathing responses, much less that of other aquatic animals. Other concerns include: (1) To what extent will aquatic animals accommodate to sustained low gravity? (2) Will test animals need induced gravity in order to function appropriately? (3) How often will test subjects need to be fed or replaced? (4) What type holding chamber designs need testing? (5) Are automated multispecies biosensing devices appropriate for space station monitoring?

Realizing these needs and gaining insight through previous studies, reasonable answers to these questions will be forthcoming. Given the advanced state of technology, the primary concerns and areas requiring additional research deal almost entirely with those of a biological nature.

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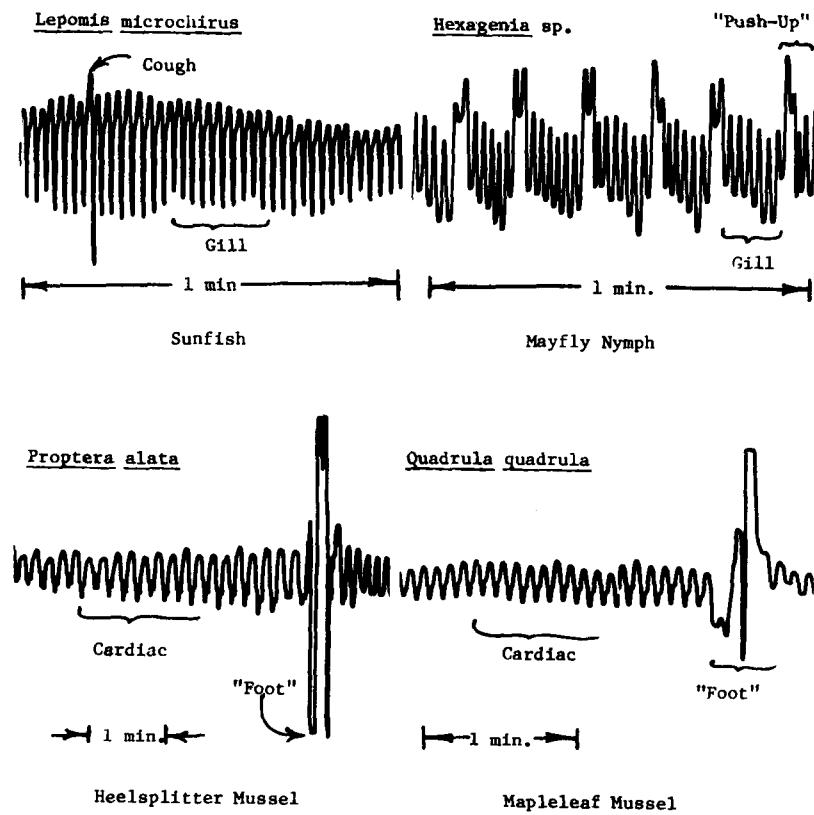


FIGURE 1. REPRESENTATIVE PHYSIOLOGICAL RESPONSES MONITORED UNDER AMBIENT WATER QUALITY EXPOSURES BY AUTOMATED MULTISPECIES BIOSENSOR AT REMOTE STREAMSIDE STATION, CENTER HILL LAKE, TENNESSEE

REFERENCE

Frequency Distribution

Group	Freq	%Freq	Cum.Freq.	%Cum.Freq.	Midpoint	Histogram
1	4	0.4	4	0.4	10.27E+00	:*
2	23	2.2	27	2.6	12.20E+00	:***
3	29	2.8	56	5.4	14.14E+00	:****
4	36	3.5	92	8.8	16.07E+00	:*****
5	60	5.8	152	14.6	18.01E+00	:*****
6	113	10.9	265	25.5	19.94E+00	:*****
7	154	14.8	419	40.3	21.88E+00	:*****
8	158	15.2	577	55.5	23.81E+00	:*****
9	131	12.6	708	68.1	25.75E+00	:*****
10	110	10.6	818	78.7	27.68E+00	:*****
11	90	8.7	908	87.3	29.62E+00	:*****
12	56	5.4	964	92.7	31.55E+00	:****
13	33	3.2	997	95.9	33.49E+00	:****
14	20	1.9	1017	97.8	35.42E+00	:***
15	5	0.5	1022	98.3	37.36E+00	:*
16	9	0.9	1031	99.1	39.29E+00	:*
17	3	0.3	1034	99.4	41.23E+00	:
18	3	0.3	1037	99.7	43.16E+00	:
19	0	0.0	1037	99.7	45.10E+00	:
20	2	0.2	1039	99.9	47.03E+00	:

TEST

Group	Freq	%Freq	Cum.Freq.	%Cum.Freq.	Midpoint	Histogram
1	24	5.3	24	5.3	16.78E-01	:*****
2	11	2.4	35	7.8	50.33E-01	:***
3	9	2.0	44	9.8	83.87E-01	:***
4	9	2.0	53	11.8	11.74E+00	:***
5	8	1.8	61	13.6	15.10E+00	:***
6	10	2.2	71	15.8	18.45E+00	:***
7	7	1.6	78	17.4	21.81E+00	:**
8	18	4.0	96	21.4	25.16E+00	:*****
9	53	11.8	149	33.2	28.52E+00	:*****
10	60	13.4	209	46.5	31.87E+00	:*****
11	48	10.7	257	57.2	35.23E+00	:*****
12	58	12.9	315	70.2	38.58E+00	:*****
13	50	11.1	365	81.3	41.94E+00	:*****
14	36	8.0	401	89.3	45.29E+00	:*****
15	16	3.6	417	92.9	48.65E+00	:****
16	13	2.9	430	95.8	52.00E+00	:****
17	14	3.1	444	98.9	55.36E+00	:****
18	4	0.9	448	99.8	58.71E+00	:*
19	0	0.0	448	99.8	62.07E+00	:
20	1	0.2	449	100.0	65.42E+00	:

Fisher's Least Significant Difference Test

LSD	Difference	Test Result
.816044	8.504669	SIGNIFICANT

FIGURE 2. BREATHING RESPONSE OF BLUEGILL SUNFISH SUBJECTED TO SUBTLE ACIDIFICATION

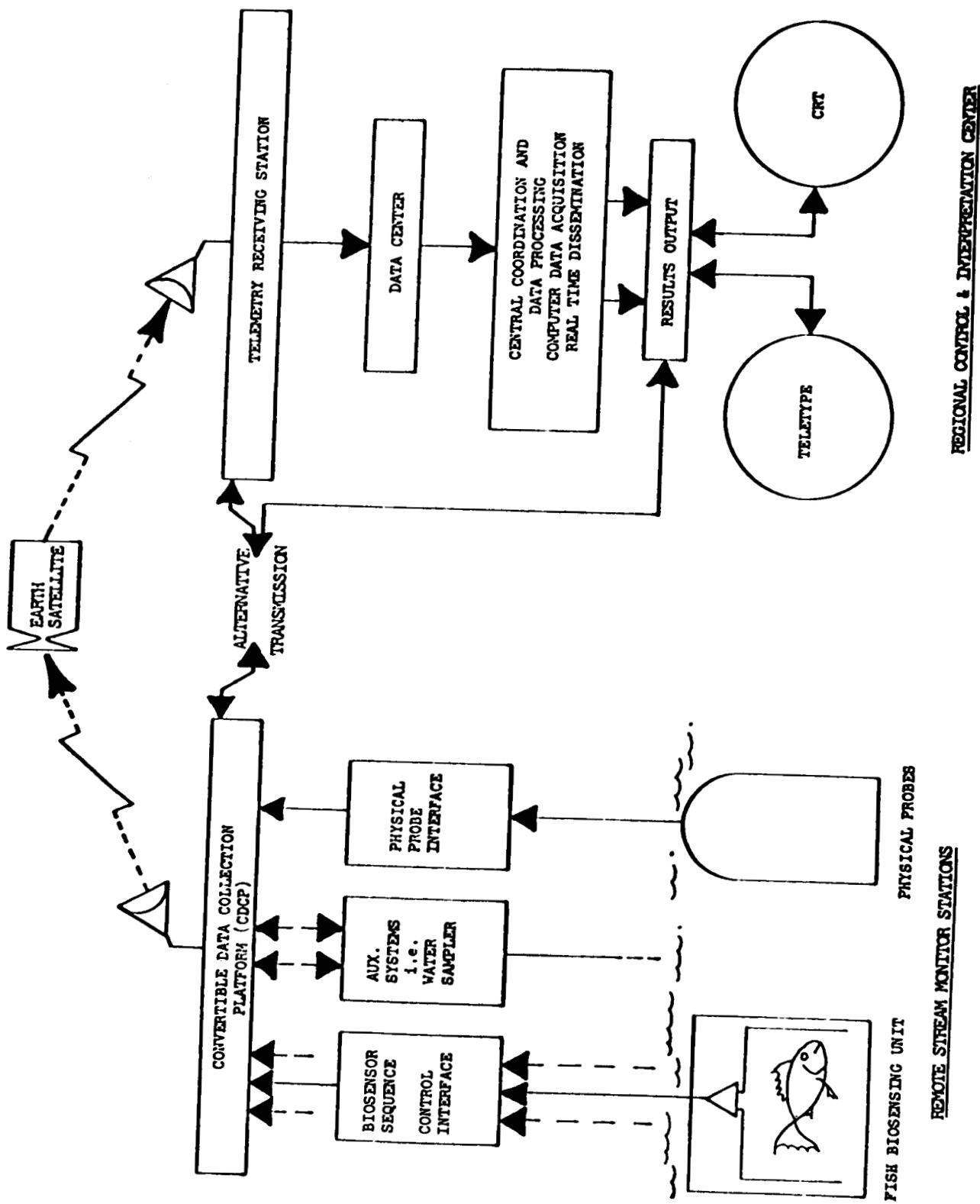


FIGURE 3. CONFIGURATION FOR SATELLITE DATA TRANSMISSION FROM REMOTE BIOMONITOR